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Pulmonary Clearance and Inflammatory Response in C3H/HeJ Mice after Intranasal Exposure to *Pseudomonas* spp.

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The environmental release of engineered microorganisms has caused health and environmental concerns. In this study, an animal model was used to examine health effects following pulmonary exposure to environmental and clinical isolates. In order to rule out the possibility that an adverse response was caused by endotoxin, 50% lethal doses (LD_{50}) were determined, when possible, with endotoxin-sensitive (C3HeB/FeJ) and endotoxin-resistant (C3H/HeJ) mice by using both environmental isolates (*Pseudomonas aeruginosa* BC16, BC17, BC18, and AC869 and *Pseudomonas maltophilia* BC6) and clinical isolates (*P. aeruginosa* PAO1 and DG1). The LD_{50} of strains AC869, DG1, and PAO1 are 1.05×10^7, 6.56×10^6, and 1.02×10^7 CFU, respectively, in C3HeB/FeJ mice and 1.05×10^7, 1.00×10^7, and 2.75×10^6 CFU, respectively, in C3H/HeJ mice. Strains BC17 and BC18 were not lethal to the animals. On the basis of the LD_{50} data, an appropriate sublethal dose (approximately 10^6 CFU) was selected. Animals were challenged intranasally with microorganisms, and clearance from the lungs and nasal cavity was determined. Strains BC17, BC18, and AC869 were not detected in lungs or nasal washes 14 days following treatment. Strains BC6, BC16, and DG1 were recovered from the nasal cavities at the end of the experiment. Only strain PAO1 was detected in lungs and in nasal cavities 14 days after treatment. At selected intervals following treatment, the percentages of polymorphonuclear leukocytes and lymphocytes in bronchoalveolar lavage samples were determined. *P. aeruginosa* AC869, PAO1, and DG1 elicited a relatively strong inflammatory response which was indirectly related to lung clearance. An intermediate response was observed in the lungs of animals challenged with *P. maltophilia* BC6 and *P. aeruginosa* BC16 and BC17. A significant increase in the ratio of lung weight to body weight was observed in strain AC869-treated animals. Our data indicate that upon intranasal exposure *P. aeruginosa* AC869 has the potential to induce adverse health effects in mice, as shown by lung and nasal cavity clearance and the pulmonary inflammatory response.

Members of the genus *Pseudomonas* are used for environmental applications because of their effectiveness in biocontrol and versatile substrate utilization abilities. For example, an engineered strain of *Pseudomonas syringae* has been shown to be useful in the prevention of ice nucleation on crops (22). The delta endotoxin genes from *Bacillus thuringiensis* have been introduced into *Pseudomonas fluorescens* for more effective delivery of the pesticidal activity (29). Other pseudomonads, such as *Pseudomonas cepacia* and *Pseudomonas putida*, can be used as fungicides for winter wheat (9). Pseudomonads can metabolize a variety of compounds introduced into the environment, such as petroleum products (20). Even though most of these microorganisms are generally considered harmless soil isolates, there is potential for adverse health effects due to exposure to high concentrations of microbial products during production or environmental application (30).

Pseudomonas aeruginosa is recognized as an opportunistic human pathogen and is capable of causing disease especially in immunosuppressed hosts and patients with leukemia or cystic fibrosis (4, 5, 15, 36). *P. cepacia* is involved in nosocomial infections (24) and can colonize and cause pneumonia and septicemia in cystic fibrosis patients (33). *P. putida*, *P. fluorescens*, and *Pseudomonas maltophilia* (*Xanthomonas maltophilia*) have been shown to cause secondary infections in cancer patients and are involved in other opportunistic infections (13).

Adverse effects from pulmonary exposure to pseudomonads can be attributed to a variety of pathogenicity factors. The pathogenicity factors include extracellular toxins, such as phospholipase, elastase, and toxin A (23, 45), and adhesins, such as exoenzyme S (1) and pili (47). Alginate production can occur in isolates obtained from cystic fibrosis patients and can cause a chronic infectious state; this persistence has been linked to decreased production of virulence factors (46). Except for alginate production, there is no distinction between clinical and environmental isolates in the expression of virulence factors (28), serotypes (19), or outer membrane proteins (16).

Pulmonary exposure to endotoxin, a component of the gram-negative cell wall, also can result in negative health effects. Research has determined that endotoxin causes fever, increased permeability (3), acute bronchoconstriction (34), an influx of macrophages (27), and release of tumor necrosis factor that can cause shock and death in humans (25, 42). Endotoxin has been shown to promote translocation of microorganisms from the gastrointestinal tract to the mesenteric lymph nodes, spleen, and liver, predominantly in gnotobiotic and antibiotic-treated mice (2).

Both rat (6, 14, 18, 39) and mouse (12, 39, 41) models have been used to study health effects following pulmonary exposure to pseudomonads. Intranasal exposure and intratracheal exposure have been used in a reproducible surrogate

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TABLE 1. Doses, selective agents, and strains used in this study

Strain	Clearance dose (CFU, 10^6)	Inflammatory response dose (CFU, 10^6)	Selective agent	Reference
P. maltophilia BC6	2.60	2.00	Kanamycin[a]	11
P. aeruginosa BC16	1.21	1.00	Ampicillin[b]	11
P. aeruginosa BC17	0.76	1.20		11
P. aeruginosa BC18	0.83	1.20	Ampicillin	11
P. aeruginosa AC869	1.74	1.40	Kanamycin	8
P. aeruginosa PAO1	1.41	1.20	Ampicillin	17
P. aeruginosa DG1	1.49	1.50	Ampicillin	47

[a] Kanamycin was added to *Pseudomonas* isolation agar at a concentration of 50 μg/ml.
[b] Ampicillin was added to *Pseudomonas* isolation agar at a concentration of 50 μg/ml.

infection model for acute microbial pulmonary exposure studies in mice (37). In previous work, the endotoxin-sensitive CD-1 mouse model was used to determine health effects following intranasal exposure (12). Because endotoxin is associated with gram-negative microorganisms, it is necessary to use a model in which endotoxin-associated effects are eliminated. In this study, 50% lethal doses (LD_{50}) of environmental and clinical pseudomonad isolates were determined when possible and compared in endotoxin-resistant and -sensitive mice. In an attempt to eliminate endotoxin effects, endotoxin-resistant mouse strain C3H/HeJ was used to examine clearance and the pulmonary inflammatory response following intranasal exposure to the pseudomonads.

MATERIALS AND METHODS

Chemicals. All chemicals used in this study were reagent grade and were obtained commercially. Methoxyfluorane was obtained from Pittman-Moore, Mundelein, Ill. The antibiotics kanamycin sulfate and ampicillin (sodium salt) were purchased from Sigma Chemical Co., St. Louis, Mo.

Bacterial strains. The *Pseudomonas* spp. strains used in this study are listed in Table 1. *P. aeruginosa* BC16, BC17, and BC18 and *P. maltophilia* BC6 were isolated from a commercial product (Sybron BiChem DC1006) designed for Aroclor 1260 degradation (11). *P. aeruginosa* AC869 was engineered to utilize 3,5-dichlorobenzoate as a sole carbon and energy source and was kindly provided by A. M. Chakrabarty, University of Illinois College of Medicine, Chicago (8). *P. aeruginosa* PAO1 and DG1, both of which are known to express many of the *P. aeruginosa*-associated pathogenicity factors, were gifts from D. E. Woods, University of Calgary Health Sciences Centre, Calgary, Alberta, Canada (17, 47). Strain AC869 was grown at 30°C. All other cultures were incubated at 37°C.

Media and buffers. The strains were grown in yeast extract-tryptone broth (26) to prepare the suspensions used for administration to mice. Enumeration of these suspensions was done on yeast extract-tryptone agar (1.5%) following dilution in Dulbecco phosphate-buffered saline (PBS) (GIBCO Laboratories, Grand Island, N.Y.). The cells were concentrated, washed, and diluted in PBS. To select for the pseudomonads from lungs and nasal cavities, *Pseudomonas* isolation agar (Difco Laboratories, Detroit, Mich.) was used for growth of the pseudomonads on solid medium. This medium was prepared according to the manufacturer's instructions and was supplemented with 50 μg of kanamycin or ampicillin per ml as indicated below and in Table 1.

Animals. Thirty-day-old male strain C3HeB/FeJ (endotoxin-sensitive) and C3H/HeJ (endotoxin-resistant) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine (40). Deionized water and food (Purina Rodent Chow 5001; Purina Mills, Inc., St. Louis, Mo.) were provided ad libitum, and the mice were housed in polycarbonate cages (five mice per cage) with pine shavings. The animals were quarantined for 10 days prior to treatment. The animals were weighed on the day before treatment and daily thereafter.

Intranasal exposure. All of the animals used in this study were treated as follows. Food was withheld for 16 h prior to dose administration. The animals were anesthetized with methoxyfluorane in a desiccator jar (inside diameter, 100 mm), and 50 μl of PBS or 50 μl of a cell suspension was injected unilaterally into the nostril cavity by using a 2.54-cm, 22-gauge feeding needle. The doses used are listed in Table 1. LD_{50} was determined by treating five animals per dose (four different doses were used). LD_{50} was calculated from the slopes and y intercepts derived from a linear regression analysis of the data.

Enumeration of administered bacterial strains obtained from lungs and nasal cavities. At 3 h and 1, 2, 5, 7, 10, and 14 days following treatment, mice were sacrificed by CO_2 asphyxiation and were dissected aseptically. A small incision was made in the trachea, and a 1-in. (2.54-cm) 22-gauge feeding needle was introduced into the airway. Heavy thread was tied around the trachea at the tip of the needle to prevent leakage. A 10-ml syringe containing 5 ml of PBS was connected to the needle, and the trachea was then backflushed with PBS. The liquid was collected from the nostrils. Next, the lungs were removed and homogenized in 5 ml of PBS. The homogenate, nasal wash, and dilutions of each type of sample in PBS were plated for enumeration on *Pseudomonas* isolation agar supplemented with the antibiotics indicated in Table 1. Following incubation for 48 h and counting, representative colonies were subcultured, and antibiotic sensitivity was determined by using antibiotic disks (BBL Microbiology Systems, Cockeysville, Md.) to eliminate contaminants in the enumeration of the strain administered. For each experiment three animals per treatment per time point were used. Duplicate experiments were performed; thus, a total of six animals per treatment per time point were used.

Inflammatory response. C3H/HeJ mice were sacrificed by injecting sodium pentobarbital (150 mg/kg) intraperitoneally at 1, 2, 5, 7, and 14 days following treatment, and cells were collected from the lungs by bronchoalveolar lavage. Each trachea was cannulated, and the lungs were flushed three times with warm saline (35 ml/kg). Cytospin preparations were stained with Diff-Quick (Baxter Diagnostics, Inc., McGaw Park, Ill.), and the percentages of alveolar macrophages, polymorphonuclear leukocytes (PMNs), and lymphocytes were determined by examining 200 representative cells. The inflammatory index was defined as the sum of the percentage of PMNs and the percentage of lymphocytes. We used four or five mice for each treatment-time combination.

Statistical analysis. A three-way analysis of variance was performed to determine what effects treatment, time, and experimental replicate had on animal weights, lung weights, and the ratio of lung weight to body weight (35). Values were considered significant if P was <0.005.

RESULTS

Overt health effects following intranasal exposure of endotoxin-resistant and -sensitive mouse strains. Following treatment, animals were observed for signs of illness, including weight loss, scruffy fur, lethargy, and conjunctivitis. Because the food was removed 16 h prior to dose administration, all groups lost weight initially. After 1.48×10^9 CFU of *P. aeruginosa* BC16 was administered to C3HeB/FeJ mice, two animals died. The weights of the survivors increased after 2 days. In C3H/HeJ mice, a weight increase occurred after the day on which the dose was administered. None of the strain BC17- or BC18-treated animals died (doses, 5.35×10^9 and 1.5×10^9 CFU, respectively), but a similar weight recovery phenomenon occurred. The weight of C3H/HeJ animals increased immediately, but the weight of the treated C3HeB/FeJ animals increased after 4 days.

All of the mice died when animals were treated with 1.22×10^8 or 1.22×10^9 CFU of strain AC869 or with 1.14×10^8 or 1.14×10^9 CFU of strain DG1. The LD_{50} of strain AC869 was 1.05×10^7 CFU for both C3H/HeJ and C3Heb/FeJ mice. When mice were treated with 1.22×10^8 CFU of strain AC869 or 1.14×10^8 CFU of strain DG1, 100% mortality was observed. The C3HeB/FeJ mice died within 24 h, but the C3H/HeJ mice died after 3 days. However, when 1.22×10^9 or 1.14×10^9 CFU of strain AC869 or DG1 was administered, all of the C3Heb/FeJ mice died within 24 h, but the C3H/HeJ animals survived an additional 1 day. When C3HeB/FeJ mice were treated with 1.14×10^7 CFU of strain DG1, all of the animals died after 3 days, which corresponded to the time when the weight of the one surviving C3H/HeJ mouse increased. The LD_{50} of strain DG1 were calculated to be 6.56×10^6 CFU for C3Heb/FeJ mice and 1.00×10^7 CFU for C3H/H3J mice. When treated with 1.22×10^7 CFU of strain AC869, three C3HeB/FeJ mice and three C3H/HeJ mice died. The weights of the survivors increased in two (C3H/HeJ) or three (C3HeB/FeJ) days.

Endotoxin-resistant and -sensitive mice were also treated with *P. aeruginosa* PAO1 or *P. maltophilia* BC6 and observed for signs of illness. The endotoxin-sensitive mice appeared to be more resistant to the treatments. Weight loss that led to death was always accompanied by scruffy fur and lethargy. No conjunctivitis was observed. When C3HeB/FeJ mice were given 1.6×10^8 CFU of strain BC6, one animal died and the weights of the survivors increased after the day on which treatment was administered. Two identically treated C3H/HeJ mice died, and a weight increase occurred after 2 days. Strain PAO1 (LD_{50}, 1.02×10^7 CFU for C3Heb/FeJ mice and 2.75×10^7 CFU for C3H/HeJ mice) was lethal within 24 h following treatment with 8.7×10^8 CFU. When C3H/HeJ mice were treated with 8.7×10^7 CFU of strain PAO1, all of the animals were dead after 24 h, whereas it took 3 days for death to occur in all of the C3HeB/FeJ animals. All of the C3H/HeJ mice that received 8.7×10^6 CFU died within 3 days following treatment, but only two of the C3Heb/FeJ mice died. The weights of the remaining mice started to increase after day 4.

Clearance of administered strains from lungs and nasal cavities. A nonlethal dose of each strain was administered individually to C3H/HeJ animals, and then the mice were monitored for 14 days following treatment (see Table 1 for the doses used). Lung and nasal cavity contents were examined. Only strain PAO1 was detected in both lungs and nasal washes 14 days after treatment (Fig. 1). *P. maltophilia* BC6 and *P. aeruginosa* BC16 and DG1 were recovered only from the nasal cavities at the end of the experiment (Fig. 1). *P. aeruginosa* BC17, BC18, and AC869 were not observed in either lung or nasal cavity washes 14 days following treatment (Fig. 1).

Pulmonary inflammatory response. One day following treatment and at intervals thereafter for 14 days, cells were collected from the lungs by bronchoalveolar lavage. The sum of the percentage of PMNs and the percentage of lymphocytes was determined. A strong response (or high response index) was observed in animals treated with *P. aeruginosa* AC869, PAO1, and DG1 (Fig. 2C). An intermediate response was observed in mice treated with strains BC6, BC16, and BC17 (Fig. 2B). No response was observed in strain BC18-treated animals or PBS controls (Fig. 2A). In general, the inflammatory responses consisted mainly of PMNs (data not shown).

Effect of treatment on body and lung weights. At the time of sacrifice, the animals were weighed and their lungs were removed and weighed. An initial decrease in body weight was observed for all animals because of the 16-h fasting period prior to treatment. However, no treatment effect on body weight was observed when data were compared with data for PBS-treated controls. A statistical analysis revealed that treatment with strain AC869 (overall) and treatment with strain BC6 (3 h only) had an effect on lung weight. In order to take body weight into account because lung weight is a function of total body weight, statistical analyses were performed on ratios of lung weight to body weight. Similar results were obtained. Treatment with strain AC869 caused an overall significant increase in the ratio of lung weight to body weight (Fig. 3), whereas a decrease in this ratio was observed at 3 h after treatment with strain BC6.

DISCUSSION

Because biotechnology agents are released in high numbers compared with organisms normally found in the environment, there is a potential for human exposure and consequent adverse effects. However, the organisms must be able to survive the stress that occurs during application. For example, during aerosolization, bacterial survival may be impaired depending on the relative humidity, temperature, distance traveled, droplet size, and UV radiation (32, 43). After accidental release or planned application, humans may ingest, inhale, or have dermal contact with engineered microorganisms (21). Illness or an allergic reaction may occur, especially if the immune system is impaired. Pulmonary response to an endotoxin is associated with an influx of erythrocytes, PMNs (which release elastase and serum components), and the release of tumor necrosis factor (25, 44). Therefore, it is not surprising that pulmonary exposure to both environmental *P. aeruginosa* isolates (BC16, BC17, and AC869) and clinical *P. aeruginosa* isolates (PAO1 and DG1) results in an influx of PMNs. Toews et al. (41) demonstrated that the cell response was challenge species specific. For example, *Staphylococcus aureus* challenge resulted in an increase in the number of alveolar macrophages, while an increase in the number of PMNs occurred after *P. aeruginosa* treatment. *Klebsiella pneumonia* caused PMN and alveolar macrophage responses. In this study, the intensity of the inflammatory response was strain dependent. Our data indicate that an environmental isolate, strain AC869, and two clinical strains, DG1 and PAO1, induced relatively strong inflammatory responses which were inversely correlated with decreases in the number of bacterial cells in the lungs.

According to the breeder, endotoxin resistance is associ-

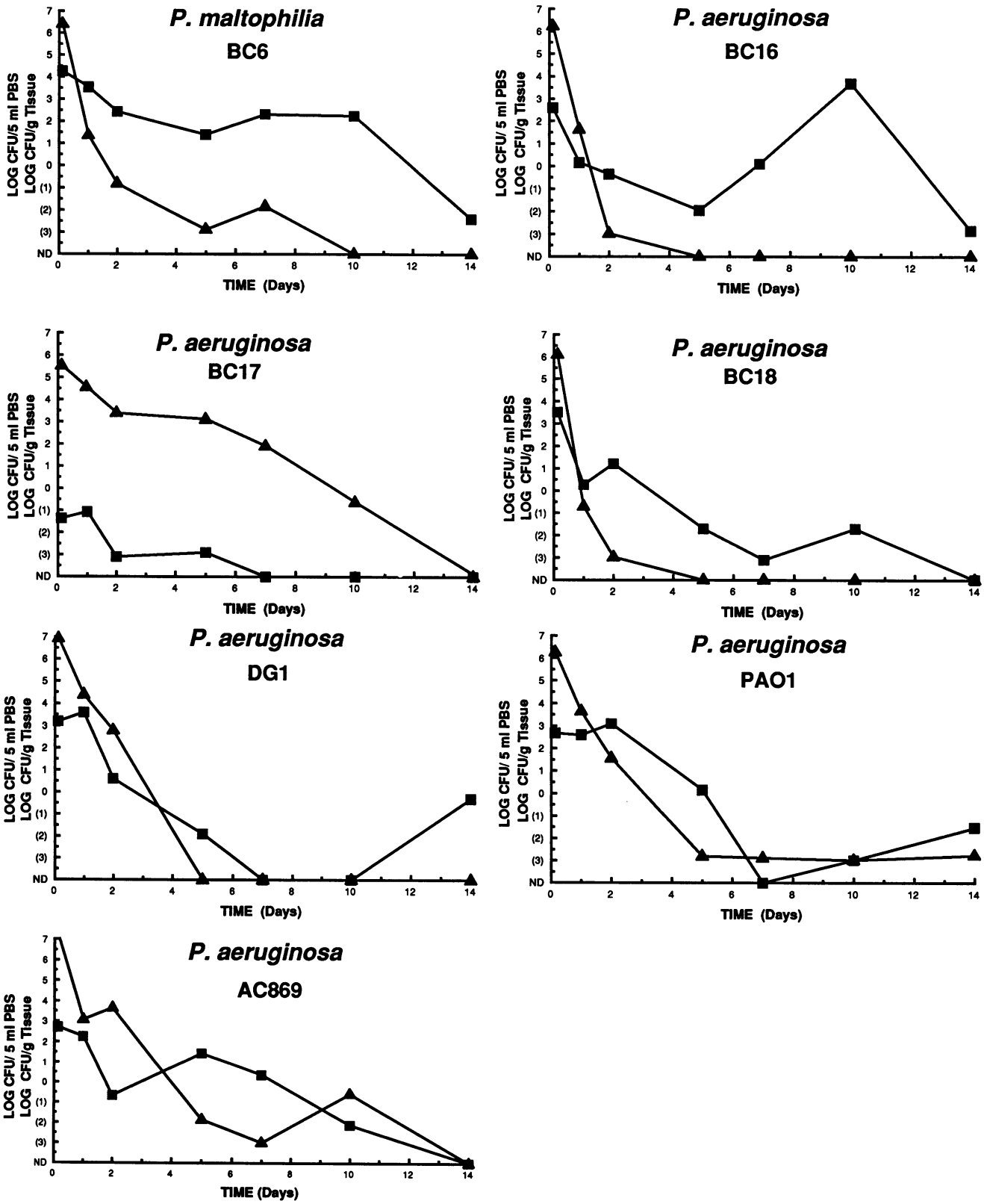

FIG. 1. Clearance of pseudomonads from lungs (▲) and nasal cavities (■) following intranasal exposure. Six mice per time per treatment were treated intranasally with each strain as described in Materials and Methods and Table 1. At different times, animals were sacrificed by CO_2 asphyxiation, the lungs were removed and homogenized, and dilutions were prepared for plating on *Pseudomonas* isolation agar; the strains were enumerated as described in Materials and Methods. The strains were recovered from nasal cavities by inserting a feeding needle into the trachea and washing the nasal cavity with 5 ml of PBS; the strains were enumerated by using the same method that was used for the lung isolates. None of the administered strains was detected in PBS-treated animals.

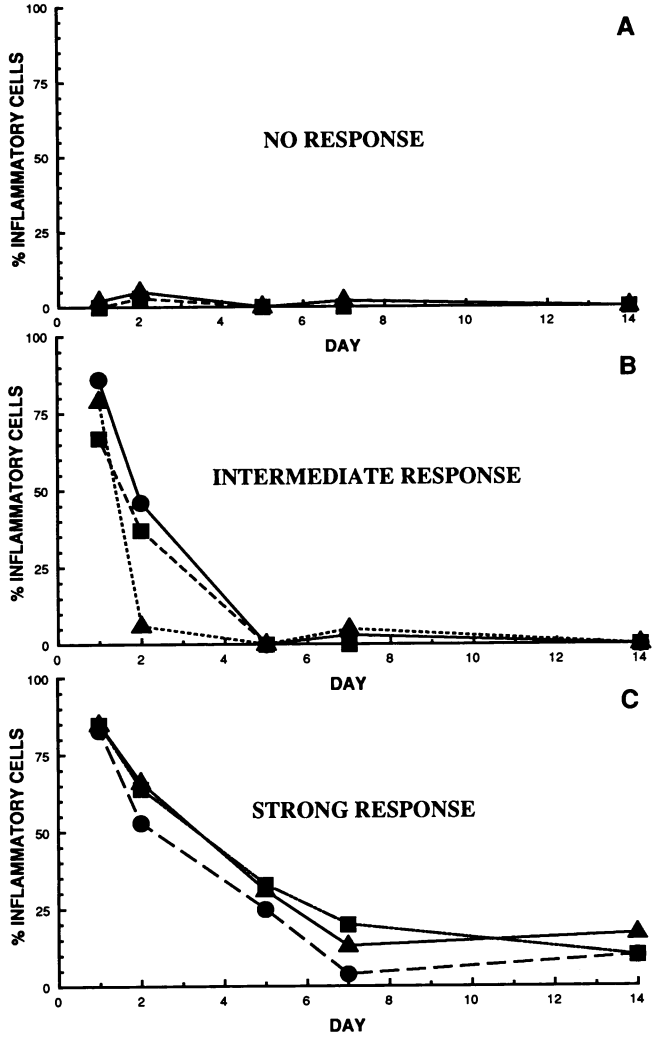

FIG. 2. Pulmonary inflammatory response index. Four or five mice were sacrificed at different times after treatment, and cells were collected from the lungs by bronchoalveolar lavage. Cytospin preparations were stained with Diff-Quick, and the percentages of alveolar macrophages, PMNs, and lymphocytes were determined by examining 200 representative cells. The inflammatory index was defined as the sum of the percentage of PMNs and the percentage of lymphocytes. (A) No response. Symbols: ■, PBS control; ▲, 1.2×10^6 CFU of *P. aeruginosa* BC18 per mouse. (B) Intermediate response. Symbols: ■, 2.0×10^6 CFU of *P. maltophilia* BC6 per mouse; ▲, 1.0×10^6 CFU of *P. aeruginosa* BC16 per mouse; ●, 1.2×10^6 CFU of *P. aeruginosa* BC17 per mouse. (C) Strong response. Symbols: ■, 1.4×10^6 CFU of *P. aeruginosa* AC869 per mouse; ▲, 1.2×10^6 CFU of *P. aeruginosa* PAO1 per mouse; ●, 1.5×10^6 CFU of *P. aeruginosa* per mouse.

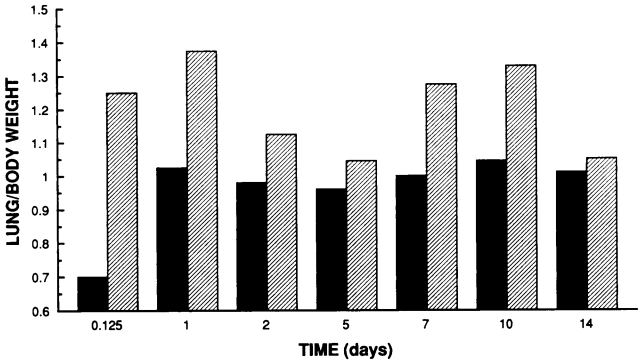

FIG. 3. Effect of *P. aeruginosa* AC869 treatment on the ratio of lung weight to body weight. Strain C3H/HeJ mice were treated with 1.74×10^6 CFU of strain AC869 per mouse (cross-hatched bars), and lungs were removed at the times indicated and weighed. The ratio of lung weight to body weight was calculated and statistically analyzed. An overall treatment effect was observed in strain AC869-challenged mice ($P = 0.0001$). The control animals (solid bars) each received 50 μl of PBS.

ated with C3H/HeJ mice (40). Therefore, endotoxin-resistant C3H/HeJ mice were used in this study to eliminate the endotoxin effects, even though there appeared to be little difference between C3HeB/FeJ and C3H/HeJ mice. Endotoxin-resistant mice appeared to be more resistant to overt endotoxin effects, such as weight loss, when they were treated with strains BC16, BC17, BC18, AC869, and DG1. However, the results of the animal treatment experiments performed with strains PAO1 and BC6 were unexpected because the endotoxin-sensitive mice (C3HeB/FeJ) appeared to be more tolerant to challenge with these strains. It should be noted that both C3H/HeJ and C3HeB/FeJ mice died when they were treated with the higher concentrations of *P. aeruginosa* AC869 (1.22×10^8 and 1.22×10^9 CFU), PAO1 (8.7×10^7 and 8.7×10^8 CFU), and DG1 (1.14×10^8 and 1.14×10^9 CFU).

Clearance of pseudomonads from lungs has been shown to be strain, host, and dose dependent. Progressive clearance, multiplication followed by clearance, or persistence can occur following challenge with *P. aeruginosa* (38). For example, when BALB/c mice were treated with 8×10^4 CFU of a *P. aeruginosa* clinical isolate, the organism was rapidly cleared from the lungs 4 h after introduction. However, at a higher dose (6×10^5 CFU) multiplication in the lungs occurred after 4 h (41). When white Swiss mice were exposed to aerosols of *P. aeruginosa* 16 (1.85×10^5 CFU deposited in lungs immediately after exposure) or *P. aeruginosa* 22 (2.65×10^5 CFU deposited in the lungs immediately after exposure), the organisms multiplied within 1 h after exposure (39). By 4 h, only 13% of the *P. aeruginosa* 16 cells remained. *P. aeruginosa* 22 persisted at a level that was 97% of the original dose. The results were different in experiments performed with rats. When 1.95×10^6 CFU of strain 16 or 4.10×10^6 CFU of strain 22 was deposited in the lungs, both strains were cleared by 4 h (the levels after 4 h were 4.9 and 5.5% of the original levels). In order to produce a chronic *P. aeruginosa* infection in rat lungs, the organism can be embedded in agar beads (6).

In this study, clearance was strain and species dependent. Progressive clearance of *P. maltophilia* BC6 occurred following microbial challenge. The organism was cleared from the lungs within 10 days, whereas *P. aeruginosa* BC16, BC17, and BC18 were not detected after 5 (BC16 and BC18) and 7 (BC17) days. Strain AC869 was cleared by 14 days after treatment. Clinical isolate DG1 was cleared from the lungs by day 5, but strain PAO1 was detected in lungs 14 days following challenge. Colonization of the nasal cavity may cause reinoculation of the lungs. For all of the strains tested, the organisms detected in the nasal cavity also were isolated from the lungs. Most of the organisms persisted longer in the nasal cavity; the only exception was strain AC869, which was present in both the lungs and nasal cavity

14 days following treatment. Strain PAO1 was not detected in the nasal cavity at day 7 and was recovered in low numbers 10 and 14 days following treatment. The level of recovery from the lungs was low as well. The apparent rebound effect may have been due to low, nondetectable concentrations of strain PAO1 in the nasal cavity followed by a regrowth phase to detectable levels at later time points. Because the bacterial counts were close to the limit of detection, animal-to-animal variation may have played a role in the inability to recover strain PAO1 on day 7.

Even though most clearance studies have been conducted with clinical isolates, several investigators have examined pulmonary exposure to environmental isolates. Goodnow et al. (14) exposed Sprague-Dawley rats to aerosols of a commercial product for ice nucleation which contained *P. syringae*. A dose-dependent increase in lung weight was observed. Kaiser et al. (18) performed a similar experiment with *P. putida* C_1 and *Pseudomonas* sp. strain CBS_a. These authors concluded that following exposure, no adverse effects were observed. Dose-dependent clearance of *P. aeruginosa* AC869 from CD-1 mice has been reported (12). In this model system, an LD_{50} of 2.7×10^7 CFU was reported. The organism was not detected 10 days after treatment, which is a shorter time than the time observed in the endotoxin-resistant C3H/HeJ mouse model. *P. cepacia* AC1100, which degrades 2,4,5-trichlorophenoxyacetic acid, caused an increase in lung weight following exposure of animals to 5.3×10^8 CFU of this organism (12); this was similar to the results obtained for strain AC869.

Adverse effects from human exposure to bacteria and endotoxins in agricultural and industrial settings have been well documented (7, 34), but there have been few studies of biotechnology products. However, in one study workers found that the endotoxin concentration in a snow inducer product (*P. syringae* in pellets) was 50,304.84 endotoxin units/ml, which was more than 100-fold higher than the concentration found in oats in a grain storage bin and in corn silage, which have been linked to respiratory dysfunction (10, 31). In addition to the endotoxin hazard, the bioengineering process may alter the expression of virulence factors, resulting in a strain that is more or less pathogenic. Consequently, there may be a potential for adverse health effects due to exposure to biotechnology products. Our results indicate that clearance from lungs, animal illness, and death are strain dependent.

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